

# ICSG 2011

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Structural and functional genomics of a model organism  
*Thermus thermophilus* HB8: toward functional discovery  
of functionally unknown proteins  
(Poster 135)

Akeo Shinkai  
Team Leader, SR system Biology Research Group  
(Group director: Dr. Seiki Kuramitsu)

RIKEN SPring-8 Center, JAPAN

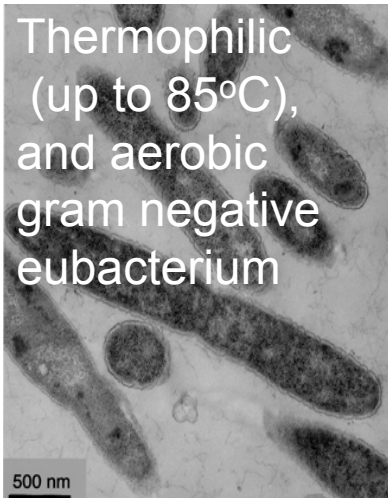
## Topic

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1. Whole Cell Project of *T. thermophilus* HB8
2. Structural Genomics
3. Functional Genomics
4. Resource and Database

# Whole cell project of *T. thermophilus* HB8

The ultimate goal of this project is to understand all of the fundamental biological phenomena at an atomic resolution, firstly, focusing on proteins.



Thermophilic  
(up to 85°C),  
and aerobic  
gram negative  
eubacterium

The reasons why *T. thermophilus* are  
(1)~2.1 Mb genome and ~2,200 genes  
(half of *E. coli* or *Bacillus subtilis*)

(2) It can grow in a minimum medium.

(3) Basic genetic engineering techniques on this strain  
are established. (construction of gene disruptant strain,  
expression of recombinant protein)

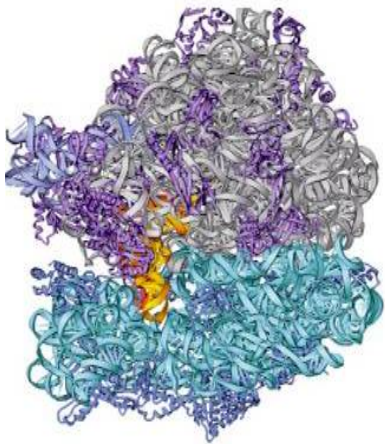
(4) Many proteins from this strain are heat stable.  
(Suitable for their structural and functional analyses)



Isolated by Dr. Tairo Oshima  
from “Mine” hot springs in Japan

# Crystal structures of large complexes and membrane proteins

Ribosome



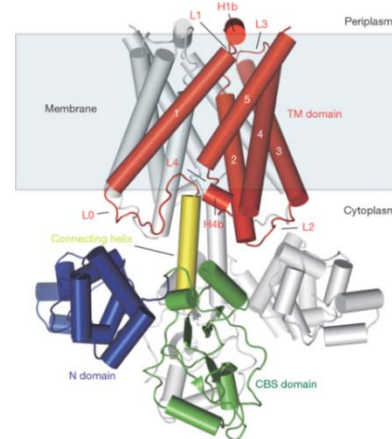
Yusupov MM *et al.* (2001) *Science* **292**, 883-896.

RNA polymerase



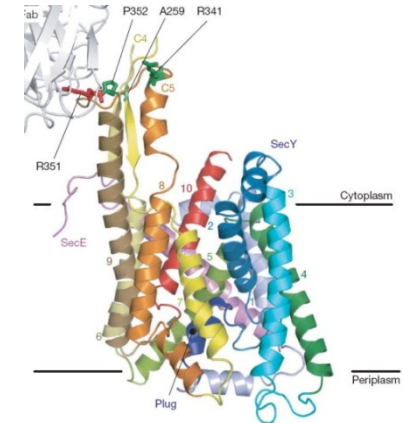
Vassylyev D *et al.* (2002) *Nature* **417**, 712-719.

MgtE Mg<sup>2+</sup> transporter



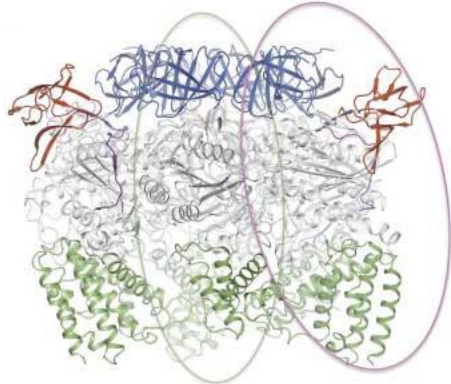
Hattori M *et al.* (2007) *Nature* **448**, 1072-1075.

SecY-SecE complex



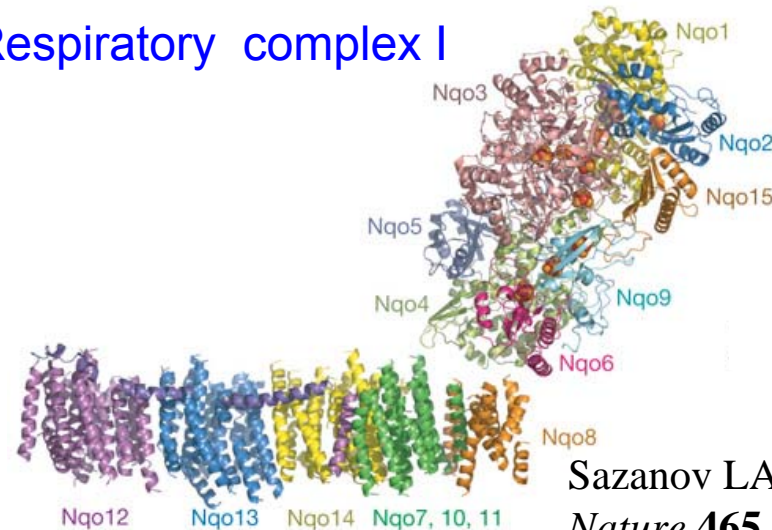
Tsukazaki T *et al.* (2008) *Nature* **455**, 988-991.

V-ATPase A<sub>3</sub>B<sub>3</sub> complex




Maher MJ *et al.* (2009) *EMBO J.* **28**, 3771-3779.

Respiratory complex I



Sazanov LA *et al.* (2010) *Nature* **465**, 441-447.



# International Structural Genomics Organization, ISGO

UserID (E-mail)

Password

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[Meetings / Conferences](#)  
[Workshop](#)  
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## Brief history of the international structural genomics projects

1995	<a href="#">Proposal of structural genomics project in Japan</a>
1997 Apr.	Start of structural genomics pilot project at RIKEN Institute
1997	Initiating study of structural genomics at DOE and NIGMS/Air
1997	<a href="#">Start of the New Jersey Initiative in Bioinformatics</a>
1997 Jan.	<a href="#">The workshop on Structural Genom</a>
1998	Start of the initial pilot projects in C
1998 Oct.	<a href="#">Structure-Based Functional Genom</a>
1999 June	Call for grant applications for NIGM

### Abstracts of the 7th Annual Meeting of the Protein Engineering Society of Japan Tokyo, May 1-2, 1995

**40. Database of heat-stable proteins from *Thermus thermophilus* HB8**

Seiki Kuramitsu, Shin-ichi Kawaguchi and Yoshiaki Hiramatsu  
*Department of Biology, Faculty of Science, Osaka University, Toyonaka, Osaka 560, Japan*

*Thermus thermophilus* HB8 is a Gram-negative eubacterium which can grow at temperatures of up to 85°C. It is the most thermophilic bacterium known to have a system of genetic engineering. Proteins from this organism are known to be thermostable and have several advantages for study as follows. (i) They are stable for a long period at room temperature and are resistant to proteases. (ii) If cloned and expressed in a mesophilic bacterium, heat treatment would be a very effective method for their purification. (iii) They have a good propensity to form crystals. Thermostable proteins from *T.thermophilus* HB8 were separated by 2-D gel electrophoresis (Kawaguchi and Kuramitsu, 1995). In all, ~1200 spots were detected on the gel with silver staining between pH values of 3 and 10. Using comigrated standard marker proteins, the molecular weight and isoelectric point of each protein spot were calculated. The average molecular weight and isoelectric point values were estimated to be 30 000 and 5.2, respectively. After the protein spots had been electroblotted onto a polyvinylidene difluoride membrane, the N-terminal amino acid sequences were determined for ~40 of them. Homologies with sequenced proteins were searched against protein databases. The information on amino acid sequences and their homologies will be useful for obtaining stable proteins and for identifying open reading frames determined from the genome DNA sequence. We are also constructing a genome DNA bank of *T.thermophilus* HB8. The genome is ~1.8 Mbp in size, and is probably one of the smallest genomes possessed by an autotroph. This small genome would thus be useful for analyzing the structure-function relationships of all the molecules in a single specified autotrophic cell. Therefore, *T.thermophilus* would be a better candidate for studying the molecular biology of an autotrophic living cell at the molecular and atomic levels. Such studies would be useful for future research in biochemistry and cell biology.

**Reference**  
 Kawaguchi,S. and Kuramitsu,S. (1995) *Electrophoresis*, **16**, 1060-1066.

**Genome decoding project (Yokoyama T & Shibata T)**

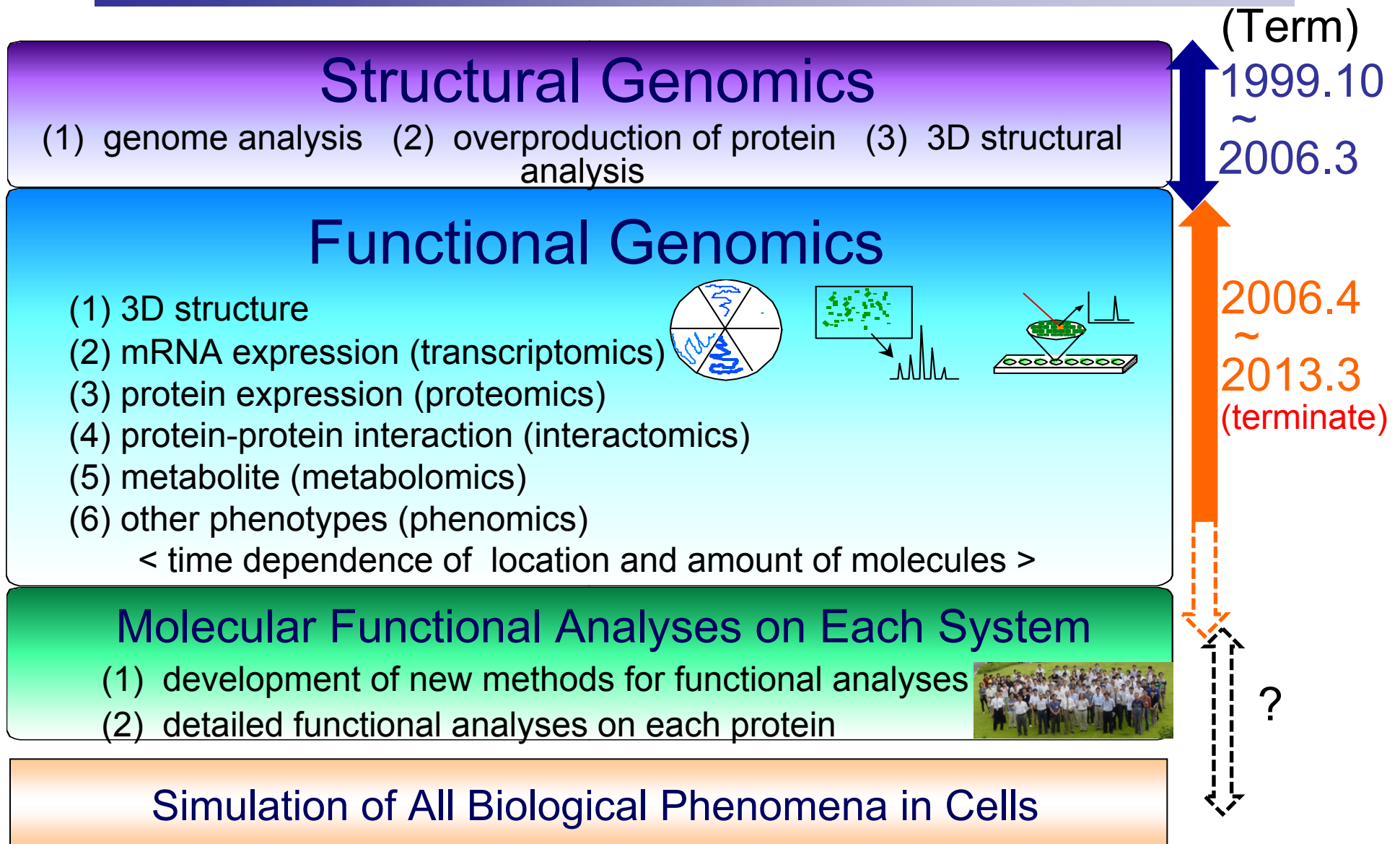
**Whole cell project (Kuramitsu S)**

## <Whole cell project in RIKEN>

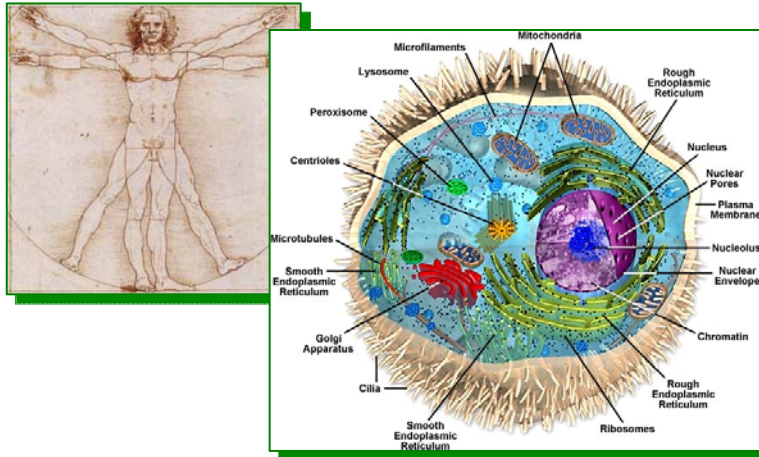
1. **Structurome Research Group, FY1999 ~ 2006**  
 (Group director: Kuramitsu S & Yokoyama S) (**\$ 2 million/year**)
2. **RIKEN Structural Genomics/Proteomics Initiative, 2001**  
 National Project on Protein Structural and Functional Analyses,  
 “Protein 3000”, FY2002 ~ 2006
3. **SR System Biology Research Group, FY2006 ~ 2012 (terminate)**  
 (Group director: Kuramitsu S.) (**now \$ 1 million/year**)



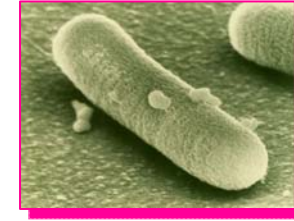
# Long-term strategy of the Whole-cell project in RIKEN



# Structural and functional genomics of *T. thermophilus* HB8



Human cell



*T. thermophilus* HB8 cell

Genes (base pairs)	23,000 ( $3 \times 10^9$ bp)	2,200 ( $2.3 \times 10^6$ bp)
Proteins	> 1,000,000 (including post-translational modifications)	2,300

With this model organism, we hope that basic biological phenomena common to many organisms, including human will be elucidated.

## Topic

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1. Whole Cell Project of *T. thermophilus* HB8
- 2. Structural Genomics**
3. Functional Genomics
4. Resource and Database



# Structure determination of the proteins

□ Genome analysis

**Genes (Proteins)**

**2,238**

Chromosome 1,849,051 bp

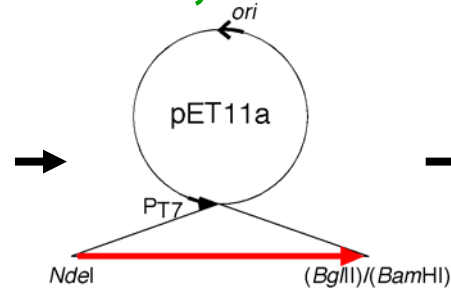
Megaplasmid (pTT27 homolog) 256,992 bp

Miniplasmid (pTT8) 9,658 bp

Total 2,115,701 bp (G+C: 69.5%)

□ Expression plasmid construction

**2,050**

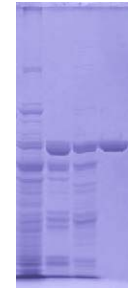


□ Overproduction in *E. coli*  
**~1,250**



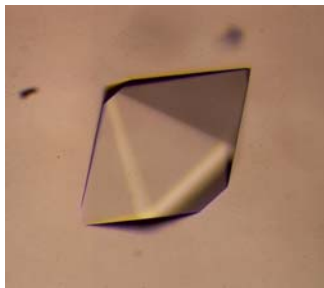
□ Purification

**~950**



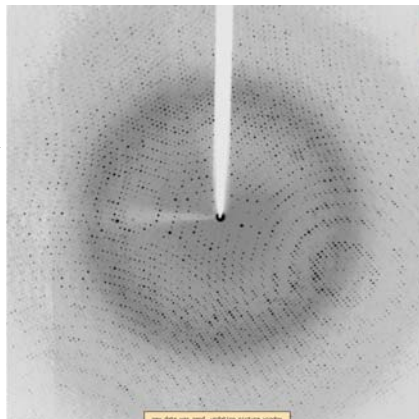
□ Crystallization

**~680**

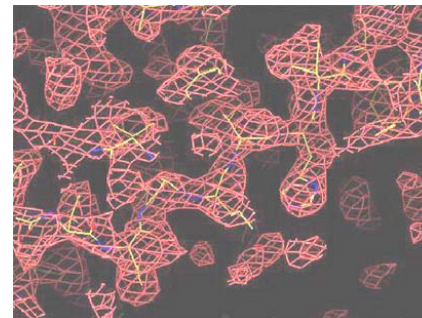


□ X-ray diffraction  
**Resolution < 2.5 Å**

**~460**



□ Calculation



□ Structure

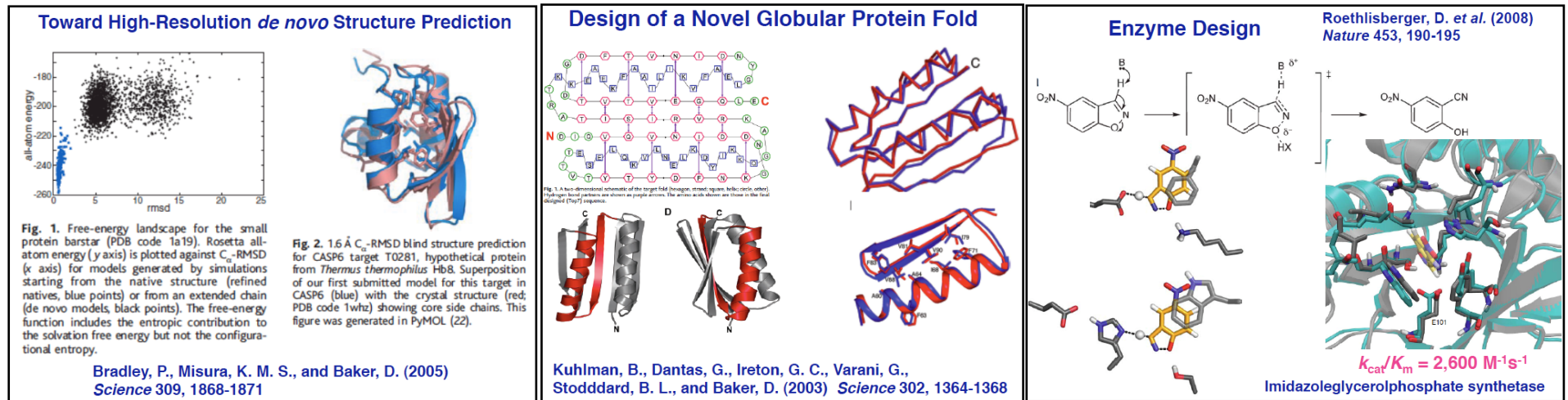
**~491** (381+ ~110)  
(including ~110 determined by the other groups)

**~22% of total**



This strain is one of the organisms whose structural genomics are much progressed.

# Prediction and *de novo* design of protein structures

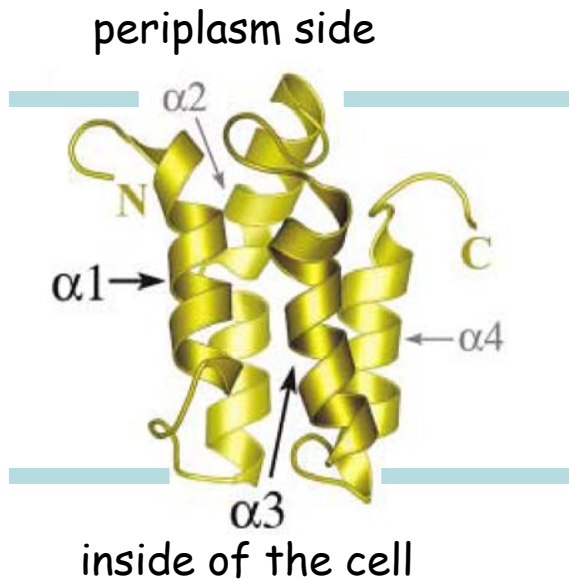


Internationally cooperative efforts in protein structure determination increased the success rate of the protein backbone conformations to about 70%.

The *T. thermophilus* protein structures also contribute to the development of programs for prediction or *de novo* design of protein structures.

# Trial expression of membrane protein

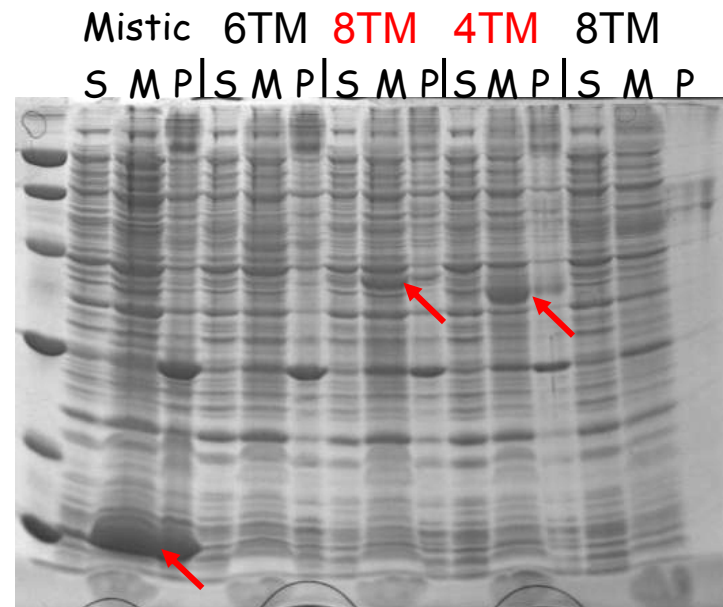
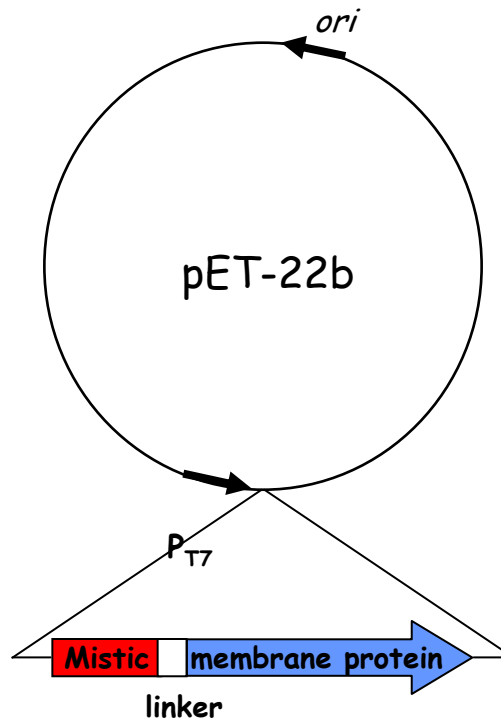
~30% of the total proteins of this organism are membrane proteins.



**Mistic** (membrane-integrating sequence for translation of integral membrane protein constructs; 110 aa) of *Bacillus subtilis*

*Signalpeptide-less membrane protein*

[Roosild, T. P. *et al.* (2005) *Science* **307**, 1317-1321]



S: soluble  
P: insoluble  
M: lauryl dimethylamine oxide soluble

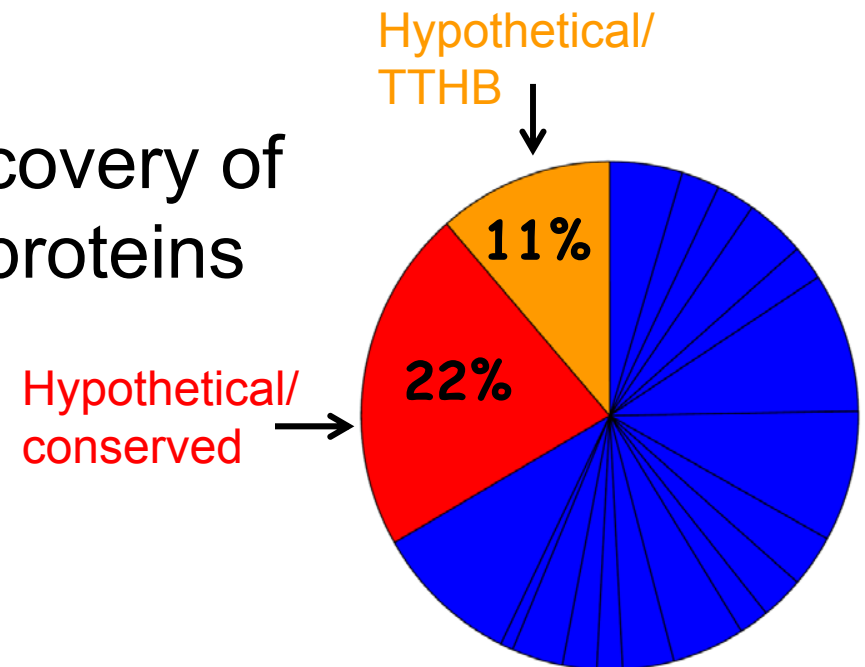
In total, nine out of 14 membrane proteins were successfully expressed by this system.

This expression system might be useful to obtain large amounts of various membrane proteins with high efficiency.

# Topic

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1. Whole Cell Project of *T. thermophilus* HB8
2. Structural Genomics
3. Functional Genomics  
~ toward functional discovery of  
functionally-unknown proteins
4. Resource and Database



“30~40% of total proteins are  
hypothetical (functionally-unknown) proteins.”

# *T. thermophilus* has many functionally unknown proteins

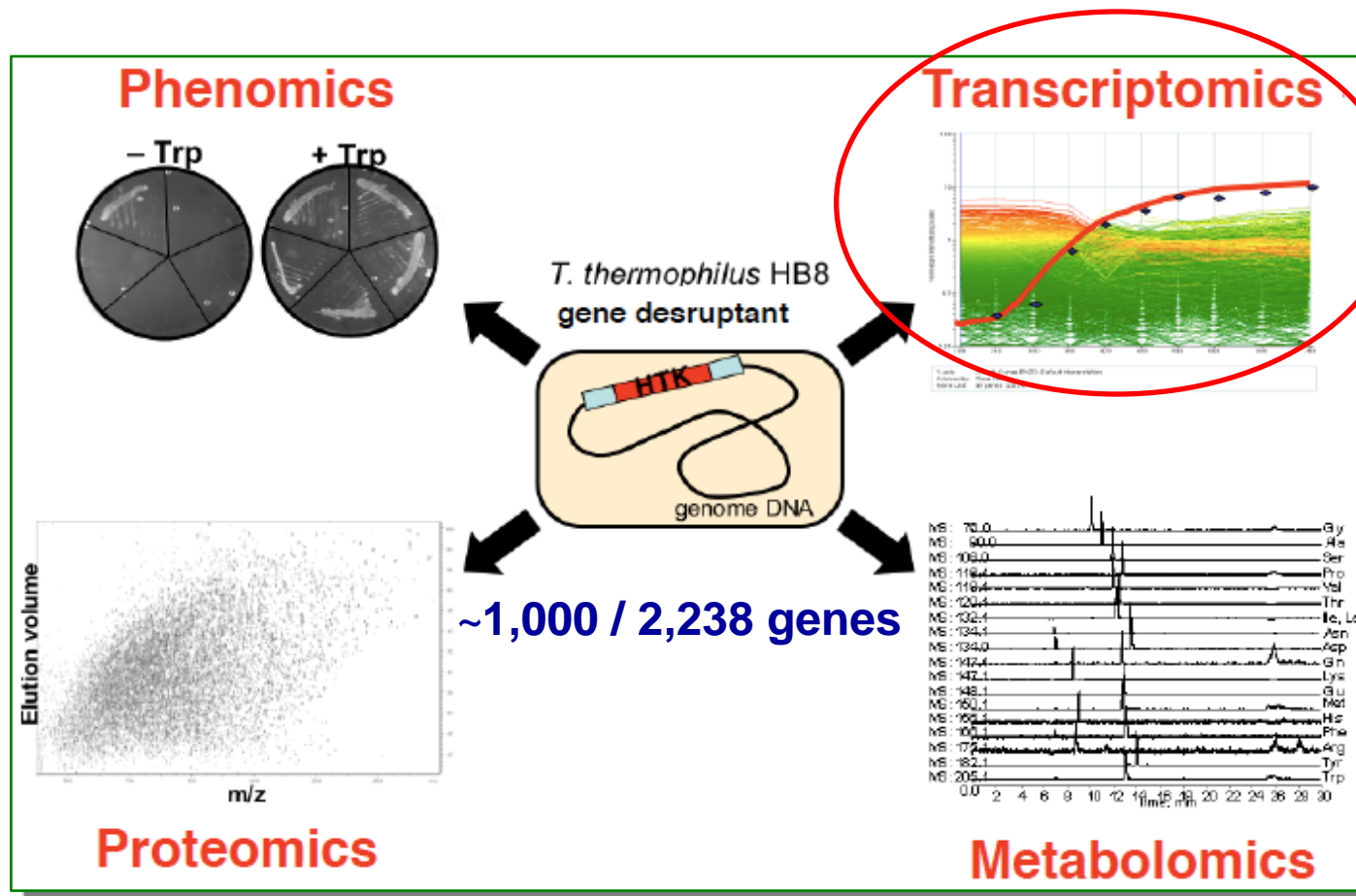
COG code	Description	No. in genome
<b>Poorly characterized</b>		
R	General function prediction only	304
<b>S</b>	<b>Function unknown</b>	<b>166</b>
<b>-</b>	<b>Not in COGs</b>	<b>434</b>

According to the **Clusters of Orthologous Group of proteins (COG)-based categorization**, **600 functionally-unknown proteins (genes)** are found in this strain.

Elucidation of function of the functionally-unknown proteins is necessary for an understanding of the whole cell life system.

Strain	COG code S	Not in COGs	Total
<i>T. Thermophilus</i> HB8	166	434	<b>600</b>
<i>E. coli</i> K-12 (W3110)	322	585	907
<i>B. subtilis</i> (str.168)	340	900	1,240

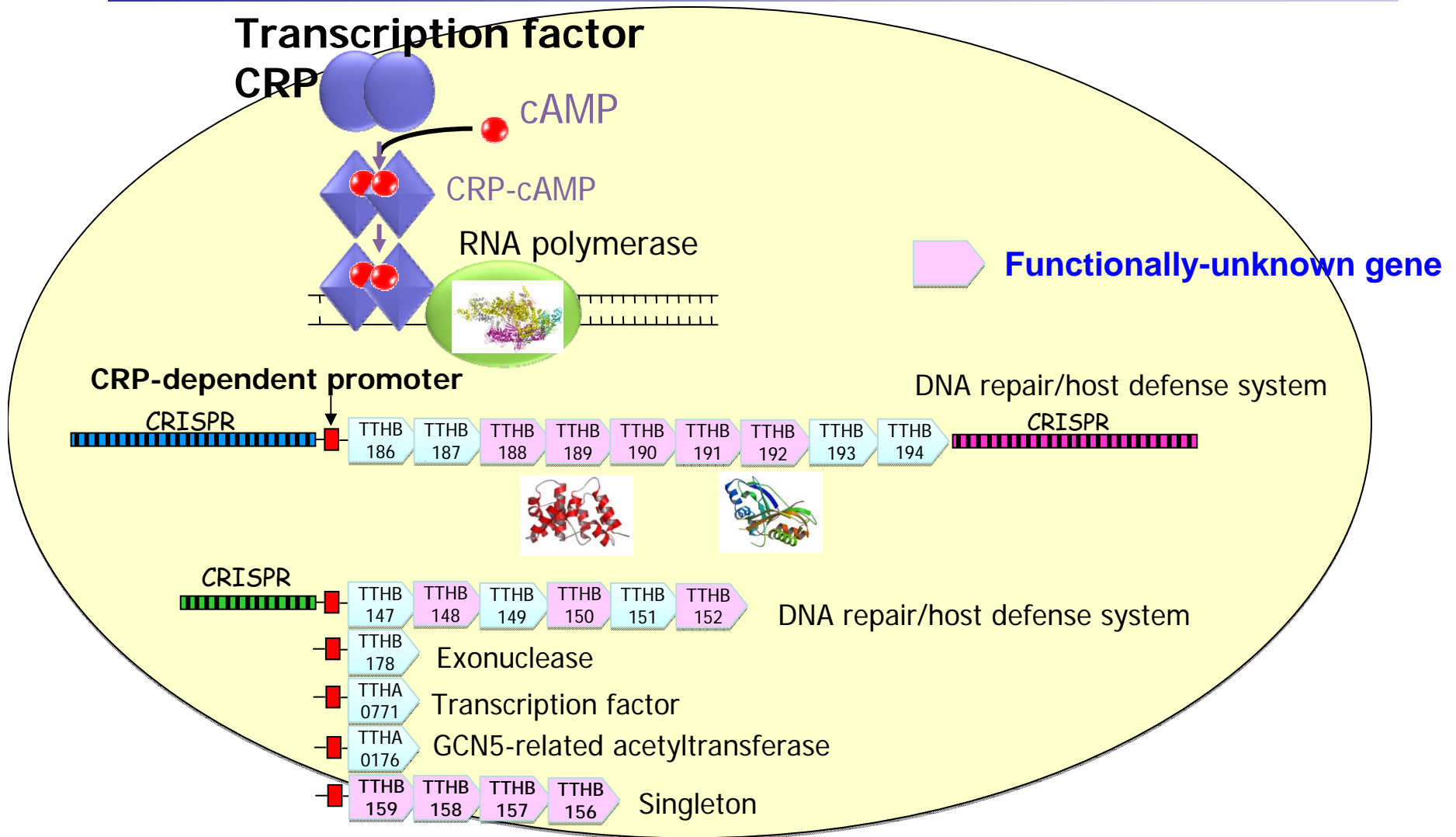
# Construction of the platforms for functomics analysis



Classify the functionally-unknown proteins (genes) based on their transcriptional regulation and obtain clues as to their function.



# Classification of the functionally unknown gene (protein) based on transcriptional regulation



Transcription of several genes sharing similar cellular function is often synchronously regulated.

## Study of transcription using *T. thermophilus* HB8

Strain	Genome (Mbp)	Number of gene	Number of transcription factor	Number of $\sigma$ factor
<i>T. thermophilus</i> HB8	2.1	2,200	~70	2
<i>Escherichia coli</i>	4.7	4,300	350	7
<i>Bacillus subtilis</i>	4.3	4,100	330	17

*T. thermophilus* HB8 is an appropriate model organism to study fundamental transcriptional regulatory system.

# Strategy for functional identification of transcription factor (TF)

## 【A】 Molecular function

### a) Identify target genes of TF

- **DNA microarray (transcriptome) analysis**

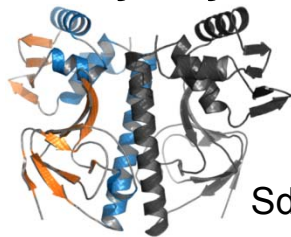
Compare total mRNA expression of TF gene-disrupted strain with that of wild type.

- **Genomic Selex**

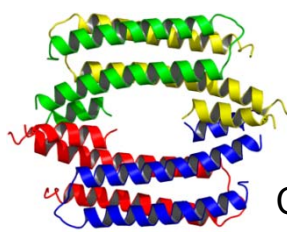
- ***In vitro* transcription analysis (and promoter search)**

### b) Determine three-dimensional structure

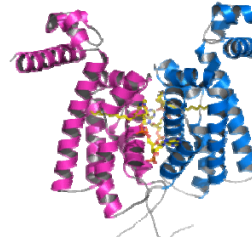
- **X-ray crystal structure**



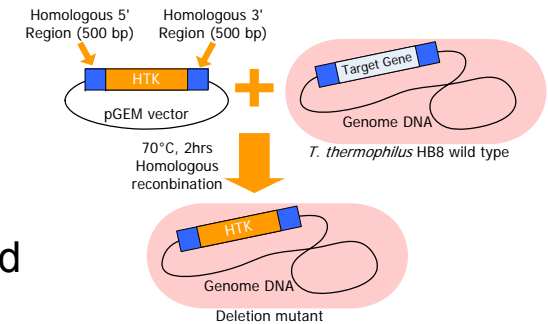
SdrP



CsoR



FadR-lauroyl-CoA



## 【B】 Cellular function (Physiological function)

### a) Analyze altered mRNA expression caused by environmental alteration

- **DNA microarray analysis**

### b) Analyze function of the target gene products (proteins)

- **Activity measurement, prediction from amino acid sequence or X-ray crystal structure**

# Summary of the number of the target gene of *T. thermophilus* regulators

18/26

Regulator	No. of the target promoter	No. of the target gene
NusG	(for the activity of RNAP)	
$\sigma^A$	(housekeeping genes)	
GreA	(for the activity of RNAP)	
Mlc	1	3
NusA	(for the activity of RNAP)	
Gfh1	(for the activity of RNAP)	
ArgR	2	5 (1)
SlrA	1	1
SlpM		




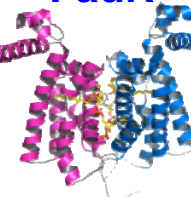
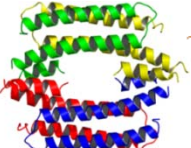
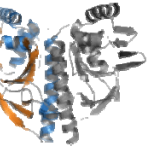



Regulator	No. of the target promoter	No. of the target gene
CRP	6	22 (12)
SdrP	16	22 (6)
FadR	9	21 (2)
PaaR	2	11 (3)
CsoR	1	3 (1)
$\sigma^E$ /anti- $\sigma^E$	3	5 (4)
TTHB099/ LitR	2	5 (0)
<b>Total</b>	<b>43</b>	<b>98 (29)</b>

 : studied by our team

( ): number of functionally unknown (COG code S or non-categorized) gene

So far, in total, 98 genes containing 29 functionally-unknown or hypothetical genes out of ~ 2,200 could be categorized based on the activity of them.

# *T. thermophilus* transcriptional regulator

	No. of protein	Protein name				
Functionally identified	18	NusG (CTD, NMR)			Gfh1	Sdr
						FadR
						
						CsoR
						
						TTHB099
						
		CRP, PaaR, $\sigma^E$ /anti- $\sigma^E$ , ArgR, SlrA, SlpM, GreA, Mlc, NusA, LitR				
		(structurally unknown)				
Functionally unknown	~46	CcpA	PyrR	Rex	Other ~43 are structurally unknown.	
						

(Blue letter: studied by our team)

Functional identification of remaining all transcription factors is necessary to classify remaining functionally-unknown proteins.

## Topic

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# Plasmids

RIKEN BIORESOURCE CENTER

[http://www.brc.riken.jp/lab/dna/en/thermus\\_en.html](http://www.brc.riken.jp/lab/dna/en/thermus_en.html)

RIKEN BIORESOURCE CENTER  
DNA BANK

Home Clone set Gene Expression Gene Analysis Search Literature

E. coli Sc. pombe S. cerevisiae T. thermophilus Other Microbe Mammalian Cells

Expression in Microbe

**Thermophilic Microorganisms Gene Plasmid**

T. thermophilus A. pernix and S. tokodaii

***Thermus thermophilus* gene plasmid**

Outline (ja) Outline (en) Expression Plasmid Disruption Plasmid Table of summary

**Outline**

*Thermus thermophilus* HB8 expression plasmids were constructed by "Whole-Cell Project of a Model Organism, *Thermus thermophilus* HB8" (<http://www.thermus.org/>) and deposited by Dr. Seiki Kuramitsu, SR System Biology Research Group, RIKEN Harima Institute; Individual clones are available.

~2,050 clones

~1,000 clones

# DNA microarray data

## NCBI Gene Expression Omnibus (GEO)

(<http://www.ncbi.nlm.nih.gov/projects/geo/>)

The screenshot shows the NCBI Gene Expression Omnibus (GEO) website. At the top, there is a navigation bar with links: HOME, SEARCH, SITE MAP, Handout, NAR 2006 Paper, NAR 2002 Paper, FAQ, MIAME, and Email GEO. Below this, a banner reads "Gene Expression Omnibus: a gene expression/molecular abundance repository supporting MIAME compliant data submissions, and a curated, online resource for gene expression data browsing, query and retrieval." The main content area is divided into three sections: QUERY, BROWSE, and SUBMIT. The QUERY section includes links to DataSets, Gene profiles, GEO accession, and GEO BLAST. The BROWSE section includes links to DataSets, GEO accessions, Platforms, Samples, and Series. The SUBMIT section includes links to Direct deposit / update, Web deposit / update, and Create new account. On the right side, there is a "Public data" section showing statistics: GPL Platforms (4951), GSM Samples (248905), GSE Series (9551), and Total (263407). Below this is a "Site contents" section with links to Documentation (Overview, FAQ, Submission guide, Linking & citing, Journal citations, Programmatic access, DataSet clusters, GEO announce list, Data disclaimer, GEO staff) and Query & Browse (Repository browser, Submitter contacts, SAGEmap, FTP site, GEO Profiles, GEO DataSets). At the bottom, there is a "Deposit & Update" section with links to Direct deposit, Web deposit, and New account. A search bar at the bottom left allows users to "Get GEO accession" with fields for Scope (Self), Format (HTML), Amount (Quick), and a "GO" button. A login section at the bottom right includes fields for User and Password, a "LOGIN" button, and a link to "Recover a password".

Platform:  
accession number GPL9209

418 samples,  
58 experimental series

# Open access homepage of the whole cell project

<http://www.thermus.org/>

The screenshot shows the homepage of the Structural-Biological Whole Cell Project. The page has a blue header with the project name and two molecular structure icons. A left sidebar contains a navigation menu with links like 'HOME', 'ABOUT', 'CONTACT', 'FAQ', 'Glossary', 'Database', 'Downloads', 'Publications', 'News', 'Links', 'Sitemap', and 'Privacy Policy'. The main content area features a 'What's New' section with a date 'Aug. 25, 2010' and a message about the 9th meeting. Below this is an 'Attention!' notice about Google Chrome. Further down is a section for 'The 9th Annual Meeting of Whole Cell Project of TTHB8'. A 'Recent 10 articles of T. thermophilus' section lists two articles. At the bottom, a section titled 'Protein expression vectors are now available from BRC' includes a link to the BRC website and a description of the clones. A red arrow points from the 'link to BIORESOURCE CENTER' text to the BRC link.

**Structural-Biological Whole Cell Project**

**What's New**

Aug. 25, 2010 Thank you very much for coming to the 9th Meeting of Whole Cell Project of TTHB8. The group photos and the abstracts are here.

**'DATABASE'**

**!Attention!**  
If you are using Google Chrome or a fast browser, please right click and select "Reload" on the pages for viewing the current pages.

**The 9th Annual Meeting of Whole Cell Project of TTHB8**

**Recent 10 articles of T. thermophilus**

Gencelver, S. et al., (2011) Crystallization and preliminary X-ray analysis of mammalian 2-phosphoglycerate phosphatase from *Thermus thermophilus* HB27. *Acta Crystallogr. Sect. F Struct Biol Cryst Commun* 67: 390-5.

Gräber, S. et al., (2011) Atomic level description of the domain closure in a dimeric protein: The case of the dimeric 2-phosphoglycerate phosphatase.

**Protein expression vectors are now available from BRC**

1685 Clones of *Thermus thermophilus* HB8 are available from RIKEN Bioresource Center (BRC).

[http://www.brc.riken.jp/lab/mna/en/thermus\\_en.html](http://www.brc.riken.jp/lab/mna/en/thermus_en.html)


All clones are constructed in pET expression system without tag residues.  
Clone set: Clones are shipped as frozen recombinant E. coli DH5a in 96-well plates with dry ice.  
Single clone: Cloned is shipped as DNA solution in TE buffer in individual tube.

**link to BIORESOURCE CENTER**

# Whole-Cell Project Database

Japanese

WCPDB - *Thermus thermophilus* HB8 Whole Cell Project Database -


Database  Target Search Mode | Article Search Mode

Genome Map chromosome (TTHA) | mega plasmid (TTHB) | mini plasmid (TTHC)

Pathway Pathway with Structure

Transcriptome datasets of *T. thermophilus* HB8 GeneChip Custom Array are available on the NCBI [GEO](#).

Accession	Subio File	Description
GPL9209	GPL9209.tab	GeneChip TTHB8401a520105F Platform Rev.2 <The new library for custom array was updated on September 18 2009 !>
GPL4902	GPL4902.tab	GeneChip TTHB8401a520105F Platform Rev.1
GSE7165		Time course of the mRNA expression in wild-type <i>Thermus thermophilus</i> HB8 strain grown on a rich medium (labeling:ENZO)
GSE7166		Comparative expression analysis between <i>crp</i> (TTHA1437) deletion mutant and wild-type of <i>T. thermophilus</i> HB8
GSE7175		SuperSeries for the study of expression analysis of the <i>T. thermophilus</i> CRP (TTHA1437)
GSE8780		Comparative expression analysis between <i>sigE</i> (TTHB211) deletion mutant and wild-type of <i>T. thermophilus</i> HB8
GSE8781		SuperSeries for the study of expression analysis of the <i>T. thermophilus sigE</i> (TTHB211)
GSE8795		mRNA expression in TTHA1939 deletion mutant of <i>Thermus thermophilus</i> HB8 strain grown on minimum medium
GSE10368	GSE10368.ssa	Time course of the mRNA expression in wild-type <i>Thermus thermophilus</i> HB8 strain grown on a rich medium
GSE10369		Comparative expression analysis between <i>sdrP</i> (TTHA1359) deletion mutant and wild-type of <i>T. thermophilus</i> HB8
GSE10370		SuperSeries for the study of expression analysis of the SdrP (TTHA1359) in <i>T. thermophilus</i> HB8
GSE10371		Comparative expression analysis between TTHB212 deletion mutant and wild-type of <i>T. thermophilus</i> HB8
GSE10548		SuperSeries for the study of expression analysis of the <i>T. thermophilus sigE</i> (TTHB211) and anti- <i>sigE</i> (TTHB212)
GSE11671	GSE11671.ssa	Comparative expression analysis between logarithmic and stationary phase of <i>T. thermophilus</i> HB8 on a synthetic medium
GSE12590		mRNA expression in TTHA0118 deletion mutant of <i>Thermus thermophilus</i> HB8 strain grown on minimum essential medium

 Subio Platform  
Free Download

FREE software for omics data management and visualization



# Whole-Cell Project Database

**Search Target**

Target: **Article** **Show All** **List View**

Species:  T. thermophilus

Locus Tag:

product: inc.

PDB: ==

☐ "OR" search ☒ "AND" search

Display: 50 Records

**Whole Cell Project Database -Target List-**

First | Prev | Records 1 - 50 / 8243 | Next | Last

Click Column Label for Change Sort Order

Locus Tag	Synonym	Product	Locus	Category	Clone for Expression	Clone for Disruption	DNA Microarray Data	Structure Status PDB	Structure Status Deposit
TTHA0001	TT0744	DNA polymerase III, beta subunit	1:55..1182	replication	available	available			
TTHA0003	TT0470	pyruvate kinase	1:2490..3914	energy metabolism	available				
TTHA0004	TT2019	hypothetical protein	1:complement(3915..4895)	hypothetical proteins	available				
TTHA0005	TT1522	metallo-beta-lactamase family protein	1:complement(4892..5506)	conserved hypothetical (ZZ)	available	available			
TTHA0006	TT0233	1-deoxy-D-xylulose-5-phosphate synthase	1:complement(5493..7340)	lipid metabolism	available				
TTHA0007	TT1523	hypothetical protein	1:complement(7349..7993)	conserved hypothetical (ZZ)	available	available			
TTHA0008	TT1141	phage shock protein A	1:complement(8037..8699)	annotated others	available	available			
TTHA0010	TT1993	hypothetical protein	1:9022..9114	hypothetical proteins					
TTHA0011	TT0257	molybdenum cofactor biosynthesis protein A (MoaA)	1:12493..13503	coenzyme biosyntheses	available				
TTHA0012	TT1913	conserved hypothetical protein, integral membrane protein	1:complement(13481..14311)	conserved hypothetical (ZZ)	available	available			
TTHA0013	TT0153	geranylgeranyl diphosphate synthetase (GGPS)	1:14329..15321	annotated others	available				1WMW 07/21/2004
TTHA0014	TT2225	hypothetical protein	1:15508..16104	hypothetical proteins					
TTHA0015	TT0238	LytB protein	1:16106..17128	lipid metabolism	available				
TTHA0016	TT1912	tRNA-dihydrouridine synthase	1:17121..18149	modification enzymes	available	available			
TTHA0017	TT1911	TPR repeat-containing protein	1:complement(18045..18677)	conserved hypothetical (ZZ)	available	available			

**T. thermophilus H88 Genome Map**

chromosome pTT27 pTT8

**Search your target**

[http://www.brc.riken.jp/lab/dna/en/thermus\\_3en.html](http://www.brc.riken.jp/lab/dna/en/thermus_3en.html)

# Whole Cell Project Database

Search Target

Target Article

Species: ^ ▾ T. thermophil

Locus Tag: ^ ▾

product: inc. ▾

PDB: == ▾

☐ "OR" search
 ☒ "AND" search

Display: 50 ▾ Records

T. thermophilus HB8 Genome Map

[chromosome](#)
[pTT27](#)
[pTT8](#)

Whole Cell Project Database -Purification-

Show All List View

**Thermus thermophilus HB8 TTHA0001**  
 DNA polymerase III, beta subunit

**Lot Information:**

<b>Purification Lot:</b>	PC010001-41-01-01
<b>Plasmid ID:</b>	PC010001-41
<b>Plasmid Vector:</b>	pET-11a
<b>Label:</b>	Native
<b>Person:</b>	Inoue Y.
<b>Purified Date:</b>	07/17/2003
<b>Total Protein Yield:</b>	42.3 mg
<b>Final Volume:</b>	2.1 mL
<b>Conc.:</b>	20.1 mg/mL

**Final SDS-PAGE:**

**Culture Lot:**

date	host cell	scale	SDS-PAGE
05/09/2003	BL21(DE3)	6 L	

**Cell Lysis and Heat Treatment:**

18.5g of <i>E. coli</i> cells
↓
Ultra Sonication with 20mM Tris-HCl + 50mM NaCl (pH8.0)
↓
Heat Treatment at 70°C for 10 min
↓
Ultra Cengrifuge at 200,000G for 1 hr

**Purification Procedure:**

Column	Buffer	Flow Rate (mL/min)	Eluted
Resource ISO 6ml	50 mM NaPi (pH 7.0), 1.2 M (NH4)2SO4	5	0.56 M AS
Resource Q 6ml	20 mM Tris-HCl (pH 8.0)	5	0.2 M NaCl
Hydroxyapatite CHT10-I	10 mM NaPi (pH 7.0)	4	50 mM NaPi
HiLoad 16/60 Superdex 75 pg	20 mM Tris-HCl (pH 8.0), 150 mM NaCl	0.5	
HiPrep 26/10 Desalting 53ml	20 mM Tris-HCl (pH 8.0)	7	